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Certified by

Jon W Dudas

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PTO/SB/16 (08-03)
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09/02/03 60/499637 00246 U.S. PTO

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EL 961800401 US

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
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Zbigniew	Pietrzkowski	San Diego, CA

Additional inventors are being named on the ______ *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Cytokinins and Cytokinin Analogs As Therapeutic Agents

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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Country		Telephone		Fax	

ENCLOSED APPLICATION PARTS *(check all that apply)*

[X] Specification *Number of Pages* 42 [ ] CD(s), Number ______

[ ] Drawing(s) *Number of Sheets* ______ [ ] Other (specify) ______

[ ] Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

[X] Applicant claims small entity status. See 37 CFR 1.27.

[ ] A check or money order is enclosed to cover the filing fees.

[X] The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 502191

[ ] Payment by credit card. Form PTO-2038 is attached.

FILING FEE Amount ($): 80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[X] No.

[ ] Yes, the name of the U.S. Government agency and the Government contract number are: ______

Respectfully submitted,

SIGNATURE [signature]

TYPED or PRINTED NAME Martin Fessenmaier

TELEPHONE 714-641-5100

Date 09/02/03

REGISTRATION NO. 46697
(if appropriate)

Docket Number: 100700.0027PRO

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

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Effective 01/01/2003. Patent fees are subject to annual revision.

[X] Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 80.00

Complete if Known	
Application Number	
Filing Date	
First Named Inventor	Dusan Miljkovic
Examiner Name	
Art Unit	
Attorney Docket No.	100700.0027PRO

METHOD OF PAYMENT *(check all that apply)*

[ ] Check [ ] Credit card [ ] Money Order [ ] Other [ ] None

[X] Deposit Account:

Deposit Account Number: 502191

Deposit Account Name: Rutan & Tucker

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FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	750	2001	375	Utility filing fee	
1002	330	2002	165	Design filing fee	
1003	520	2003	260	Plant filing fee	
1004	750	2004	375	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	80.00

SUBTOTAL (1) ($) 80.00

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

			Extra Claims		Fee from below		Fee Paid
Total Claims		-20** =		X		=	
Independent Claims		- 3** =		X		=	
Multiple Dependent						=	

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	84	2201	42	Independent claims in excess of 3
1203	280	2203	140	Multiple dependent claim, if not paid
1204	84	2204	42	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($)

***or number previously paid, if greater; For Reissues, see above*

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	410	2252	205	Extension for reply within second month	
1253	930	2253	465	Extension for reply within third month	
1254	1,450	2254	725	Extension for reply within fourth month	
1255	1,970	2255	985	Extension for reply within fifth month	
1401	320	2401	160	Notice of Appeal	
1402	320	2402	160	Filing brief in support of an appeal	
1403	280	2403	140	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,300	2453	650	Petition to revive - unintentional	
1501	1,300	2501	650	Utility issue fee (or reissue)	
1502	470	2502	235	Design issue fee	
1503	630	2503	315	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	750	2809	375	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	750	2810	375	For each additional invention to be examined (37 CFR 1.129(b))	
1801	750	2801	375	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) __________

*Reduced by Basic Filing Fee Paid

SUBTOTAL (3) ($)

SUBMITTED BY				(Complete *(if applicable)*)	
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CYTOKININS AND CYTOKININ ANALOGS AS THERAPEUTIC AGENTS

Field of The Invention

Use of cytokinins as therapeutic agents in the treatment of diseases responsive to administration of cytokinins, and especially to diseases associated with AMP (adenosine monophosphate)-activated protein kinase (AMPK), and/or pathways associated with AMPK.

Background of The Invention

Cytokinins have long been known as a relatively large class of molecules that affect growth and differentiation of plant tissue, and retard ageing of plants. Further reported *in vivo* functions of cytokinins include enlargement and/or formation of flowers and fruits, and yet other functions of selected cytokinins includes their influence on the appearance of cell organelles, and the flow of assimilates and nutrients through a plant. Moreover, various cytokinins were also reported to enhance a plant's resistance to adverse environments. Therefore, many cytokinins are employed as important tools in treating various plants, fruits, and/or seeds in agriculture and horticulture.

Seminal work on cytokinins was done by Skoog, Hecht, and others, and various cytokinins and their functions are described in "Cytokinins", *Annual Review of Plant Physiology*, Vol. 21, 1970, pages 359-383, by Skoog and Armstrong; in "Cytokinins: Syntheses, Mass Spectra, and Biological Activity of Compounds Related to Zeatin", *Proceedings of the National Academy of Science*, Vol. 63, No. 1, 1969, pages 175-185, by Leonard, Hecht, Skoog and Schmitz; in "Cytokinins Influence of Side-Chain Planarity of N^6-Substituted Adenines and Adenosines On Their Activity in Promoting Cell Growth", *Phytochemistry*, Vol. 9, 1970, pages 1907-1913, by Hecht, Leonard, Schmitz and Skoog; and in "Cytokinins: Structure/Activity Relationships", *Phytochemistry*, Vol. 6, 1967, pages 1169-1192, by Skoog, Hamzi, Szweykowska et al, and *in "Cytokinins. New Insight into a Classic Phytohormone", Vol. 128, 2002, pages 354-362 by Haberer and Kieber,* all of which are incorporated herein by reference.

Despite the diverse utility of numerous cytokinins in plants, use of cytokinins in mammals, and especially human, is limited. To the best of our knowledge, the only example of

such an application is in skin anti-wrinkle creams containing kinetin, which is based on the publication: "Kinetin delays the onset of ageing characteristics in human fibroblasts", *Biochem. Biophys. Res. Commun.*, Vol. 201, (1994), pp 665-672, by Suresh I.S. Rattan and Brian F.C.Clark, which is incorporated by reference herein. However, the mechanism of kinetin activity in human fibroblasts has not been established. Other observed biological activities of cytokinins were limited to cell culture systems and specifically related to growth inhibitory, anti-tumor, and anti-transformation effects in human lymphocytes, Erlich ascites tumor cells, leukemic myoblasts, fibroblasts, mouse sarcoma cells and 3T3 cells. However, none of these observations have apparently led to a therapeutic treatment of neoplastic diseases.

With respect to their chemical structure, cytokinins constitute a relatively diverse class of molecules. For example, compounds with cytokinin activity include numerous substituted purine and pyrimidine type compounds (*e.g.*, N^6-substituted adenine or N^4-substituted cytidine), various substituted phenylurea-type compounds (*e.g.*, (2-chloro-4-pyridyl)-N^2-phenylurea), various substituted thiourea derivatives (*e.g.*, 1,1-epsilon-polymethylenebis-(3-arylsubstituted)thioureas), and further compounds listed elsewhere herein.

The molecular mechanism of cytokinins in plants was only recently characterized and it is now believed that a specific cytokinin receptor CRE1 recognizes and binds a cytokinin, and that the CRE1 polypeptide has histidine kinase activity (see *e.g.*, Schmulling in *Trends Plant Sci.* (2001), 6(7): 281-4, or Inoue et al. in *Nature* (2001), 409 (6823):1060-3), which is thought to be associated with phosphorelay signaling in the plant cell (see *e.g.*, Hwang, et al. in *Nature* (2001), 413(6854): 383-9). Interestingly, there appears to be no significant homology between the kinases CRE1 and AMPK.

AMPK is a heterotrimeric protein with one alpha subunit having catalytic activity, and one non-catalytic beta and gamma subunit, in which the alpha subunit typically requires phosphorylation for full kinase activity. Mammalian AMPK is considered to be related to the *S. cerevisiae* Snf1 protein kinase, which is involved in various responses to nutritional stress. Similarly, the mammalian noncatalytic beta and gamma subunits are related to yeast proteins that interact with Snf1: the beta subunit to the Sip1/Sip2/Gal83 family of transcription regulators, and the gamma subunit to Snf4, which is thought to be an activator of Snf1 (see *e.g.*, Roles of the

AMP-activated Snf1 protein kinase family in the response to cellular stress. *Biochem. Soc. Symp.* 1999; 64:13-27).

It is generally thought that AMPK, or one or more specific AMPK-associated signaling pathways act as a "metabolic master switch", and/or as a sensor of cellular energy charge in mammalian cells. Likewise, the yeast AMPK homologue (*i.e.*, the Snf1 complex) is believed to act as a metabolic switch associated with a biological response to glucose starvation. Thus, AMPK appears to play a role in protecting cells from stress that causes ATP depletion by downregulating ATP-consuming biosynthetic pathways.

Yamauchi et al. demonstrated that phosphorylation and activation of AMPK are stimulated by globular and full-length adiponectin in skeletal muscle, and by full-length adiponectin in the liver (see *e.g.*, Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nature Med.* 8: 1288-1295, 2002). In parallel with its activation of AMPK, adiponectin is also thought to stimulate phosphorylation of acetyl coenzyme A carboxylase, fatty acid oxidation, glucose uptake, and lactate production in myocytes, phosphorylation of ACC and reduction of molecules involved in gluconeogenesis in the liver, and reduction of glucose levels *in vivo* (Adiponectin is a hormone secreted by adipocytes that regulates energy homeostasis and glucose and lipid metabolism). Blocking AMPK activation by a dominant-negative mutant inhibits each of these effects, indicating that stimulation of glucose utilization and fatty acid oxidation by adiponectin occurs through activation of AMPK. Yamauchi et al. (*ibid.*) concluded that their data supported the paradigm that an adipocyte-derived antidiabetic hormone (adiponectin) activates AMPK, thereby directly regulating glucose metabolism and insulin sensitivity *in vitro* and *in vivo*.

Consequently, it is believed that activation of AMPK directs cellular metabolism from anabolic processes toward catabolic processes to create ATP. For example, synthesis of cholesterol, fatty acid, and triglyceride synthesis may be reduced while processes such as beta oxidation, glucose uptake, and glycogenolysis are increased. Therefore, selective activation of AMPK is considered to provide numerous beneficial effects, and a few AMPK activators are known in the art.

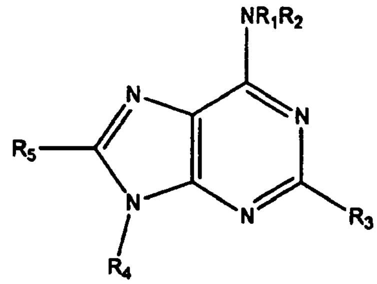
For example, the nucleoside analog AICAR is known to activate AMPK (5-Amino-4-imidazole carboxamide ribonucleotide; see *e.g.*, *Eur J Biochem.* 1995;229(2): 558-65) via a mechanism that involves intracellular conversion of AICAR to its metabolite ZMP (5-aminoimidazole-4-carboxamide 1-beta-D-ribofuranotide), which is thought to be an AMP mimic. However, activation of AMPK via AICAR is often moderate and typically requires relatively high concentration *in vivo* to achieve significant AMPK activation. In another example, metformin (N,N-dimethyldiguanide) was demonstrated to activate AMPK (see *e.g.*, *J. Clin. Invest.* 2001; 108(8): 1105-1107), and is in some cases employed as a therapeutic agent for treatment of non-insulin dependent diabetes mellitus (NIDDM). However, serious side effects (*e.g.*, lactic acidosis) may occur, especially when administered over an extended period or to elderly patients. In a further example, rosiglitazone (5-((4-(2-Methyl-2-(pyridinylamino)ethoxy)phenyl)methyl)-2,4-thiazolidinedione-2-butenedioate; see *e.g.*, *J. Biol. Chem.* 2002; 277(28): 25226-32) is known to activate AMPK to at least some extent. However, as with metformin, rosiglitazone is not always well tolerated in all patients.

Therefore, although numerous agents for AMPK activation or activation of pathways associated with AMPK are well are known in the art, one more problems remain. Therefore, there is still a need to provide improved composition and methods for treatment of conditions associated with AMPK activation or activation of pathways associated with AMPK.

Summary of the Invention

The present invention is directed to use of one or more cytokinins and/or one or more cytokinin analogs to activate AMPK, or an AMPK-associated pathway, and especially relates to the use of such compounds in the treatment of conditions and diseases associated with activation of AMPK, or activation of an AMPK-associated pathway.

Therefore, the inventors contemplate in one aspect of the inventive subject matter a pharmaceutical composition that comprises at least one cytokinin or cytokinin analog at a concentration effective to activate AMPK or to activate an AMPK-associated pathway in a mammal when the composition is administered to the mammal. Especially preferred cytokinins and cytokinin analogs will have a structure according to Formula (I)



Formula (I)

wherein R_1 and R_2 are independently H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted alkaryl, optionally substituted heteroaryl, optionally substituted heterocycle, OH, NOH, CN, or NR_3R_4, and wherein R_3, R_4, and R_5 are independently H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted alkaryl, optionally substituted heteroaryl, optionally substituted heterocycle, NH_2, OH, NOH, CN, CF_3, O-alkyl, S-alkyl, NH-alkyl, carbohydrate radical (more preferably monosaccharide radical, and most preferably furanosyl)..

Particularly preferred cytokinins and cytokinin analogs include a substituted thiourea, a N-monosubstituted urea, or N,N'-disubstituted urea (*e.g.*, N-(4-pyridazinyl)urea, or a N-(2,6-disubstituted 4-pyridyl)-N'-phenylurea), a substituted nitroguanidine (*e.g.*, substituted with an optionally substituted alkyl, alkenyl, or alkynyl radical), a substituted ethanolamine, a substituted oligo(imino-amine), or a substituted sulfonamide. It is further especially preferred that administration of such compositions is indicated for treatment of a disease associated with dysregulation of AMPK or dysregulation of an AMPK-associated pathway ((*e.g.*, non-insulin dependent diabetes mellitus, or dyslipidemia).

Thus, in another aspect of the inventive subject matter, a pharmaceutical composition may comprise at least one cytokinin or cytokinin analog in a formulation (preferably oral) for treatment of non-insulin dependent diabetes mellitus or a dyslipidemia, with the proviso that the cytokinin or cytokinin analog is a compound other than metformin, glyburide, AICAR, and rosiglitazone. With respect to suitable alternative cytokinins and cytokinin analogs, the same considerations as outlined above apply.

Consequently, a method of creating a pharmaceutical composition for administration to a human will include one step in which a compound is identified as having cytokinin activity, and another step in which the compound is incorporated into the pharmaceutical. Especially preferred steps of identifying include an AMPK kinase assay, a CRE1-binding assay, an *in vivo* or *in vitro* growth enhancement assay, and/or a correlation of the compound with one or more known cytokinin or cytokinin analogs.

In still further contemplated aspects of the inventive subject matter, a nutritional supplement will include a cytokinin and/or cytokinin analog in an amount effective to reduce blood glucose concentration, triglyceride concentration, and/or a cholesterol concentration, and it is particularly preferred that the cytokinin or cytokinin analog is present as a constituent of a plant extract (*e.g.*, barley).

Furthermore, the inventors contemplate a method of marketing a product (*e.g.*, food stuff, pharmaceutical composition, or nutritional supplement) in which in one step it is advertised that a cytokinin or a cytokinin analog reduces at least one of a blood glucose concentration, a triglyceride concentration, and a cholesterol concentration, or activates at least one of an AMPK or an AMPK-associated pathway. Again, especially preferred cytokinin or cytokinin analog may be present as a constituent of a plant extract (*e.g.*, barley), but synthetic cytokinins or cytokinin analogs are also deemed suitable.

In yet further contemplated aspects of the inventive subject matter, a pharmaceutical composition may comprise an anticytokinin in a formulation for treatment of a disease associated with an overactivity of AMPK or an overactivity of an AMPK-associated pathway, and it is especially contemplated that suitable anticytokinins comprise a 4-N-substituted-2-S-substituted pyrrolo[2,3-d]pyrimidine. Among other beneficial uses, anticytokinins may be particularly useful to treat ventricular tachyarrhythmia.

Various objects, features, aspects and advantages of the present invention will become more apparent from the following detailed description of preferred embodiments of the invention.

Detailed Description

The inventors surprisingly discovered that various compounds with cytokinin activity will significantly modulate various biological systems and/or processes in non-plant cells, and especially contemplated non-plant cells include mammalian (and especially human) cells.

Such discovery is particularly unexpected as cytokinins were heretofore recognized as specific biological effector molecules ("plant hormones") with selectivity to higher organisms of plant kingdom (and only to a very limited extent as influencing selected neoplastic mammalian cells). For example, the American Heritage Dictionary (2000, Houghton Mifflin Company) defines the term "cytokinin" as "Any of a class of plant hormones that promote cell division and growth and delay the senescence of leaves".

As used herein, the term "cytokinin" refers to a naturally occurring compound with cytokinin activity, wherein such a compound will typically (but not necessarily) include a purine or pyrimidine scaffold with one or more substituents covalently bound to a nitrogen atom (*e.g.*, N^6 in the case of a 6-aminopurine scaffold [adenine], or N^4 in the case of a 4-amino-2(1H)-pyrimidinone scaffold [cytosine]). The term "cytokinin analog" as used herein refers to a non-naturally occurring compound with cytokinin activity. The chemical structure of such cytokinin analogs may vary considerably, however, particularly preferred cytokinin analogs will include a substituted urea, a substituted thiourea, a substituted guanidine, a substituted sulfonamide, or a substituted ethanolamine (see below).

The term "cytokinin activity" as used herein refers to an activity that is characterized as a positive test result in at least one of the following test protocols:

(1) Soy bean callus culture: A positive test result is obtained when a test compound leads to an increase of at least 10% (and more typically at least 20%) in dry weight of the callus or at least 30% (and more typically at least 45%) in fresh weight of the callus as compared to a control without cytokinin in the callus growth medium. A general procedure is provided in U.S. Pat. No. 4,995,903 (Example 3).

(2) Cucumber cotyledon test: A positive test result is obtained when a test compound has an ED_{50} of less than 200. The test procedure is a modification of the protocol described in Plant Physiology (1982), 69: 695 et seq., and general procedure for the cucumber cotyledon test is provided in U.S. Pat. No. 4,995,903 (Example 2).

(3) Tobacco callus test: A positive test result is obtained when a test compound leads to an increase of at least 10% (and more typically at least 20%) in fresh weight of the callus as compared to a control. A general procedure is provided in *Journal of Biological Chemistry* (1975), 250(18): 7343-7351.

(4) Cytokinin response regulator test: A positive test is obtained when a test compounds increases at least four of six type-A response regulators in an amount of at least 10% in a test system as described by Asakura et al. in *Plant Mol Biol.* 2003 May;52(2):331-341, which is incorporated by reference herein.

(5) Alternatively, it is also contemplated that cytokinin activity may be identified by virtue of activation of AMPK, and a quantitative assay is described in *Biochem. Biophys. Res. Commun.* (1994), 200(3):1551-6 by Sullivan et al. (Characterization of 5'-AMP-activated protein kinase in human liver using specific peptide substrates and the effects of 5'-AMP analogues on enzyme activity). A positive test result is obtained when a test compound increases phosphorylation of a substrate at least 5% over control.

For further guidance, the following papers describe detection and/or measurement of cytokinin activity, all of which are incorporated by reference herein. Skoog et al. (1967) *Phytochem.* 6:1169-1192; Morris (1986) *Ann. Rev. Plant Physiol.* 37:509-538; Horgan (1984) in *Advanced Plant Physiol.* pp. 53-75; and Letham and Palni (1983) in *Ann. Rev. Plant Physiol* 34: 163-197.

The term "alkyl" as used herein refers to unsaturated hydrocarbon groups in a straight, branched, or cyclic configuration (also referred to as cycloalkyl, see below), and particularly contemplated alkyl groups include lower alkyl groups (*i.e.*, those having six or less carbon atoms). Exemplary alkyl groups are methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, tertiary butyl, pentyl, isopentyl, hexyl, isohexyl, etc. The term "alkenyl" as used herein refers to an alkyl

as defined above and having at least one double bond. Thus, particularly contemplated alkenyl groups include straight, branched, or cyclic alkenyl groups having two to six carbon atoms (*e.g.*, ethenyl, propenyl, butenyl, pentenyl, etc.). Similarly, the term "alkynyl" as used herein refers to an alkyl or alkenyl as defined above and having at least one triple bond. Especially contemplated alkynyls include straight, branched, or cyclic alkynes having two to six total carbon atoms (*e.g.*, ethynyl, propynyl, butynyl, pentynyl, etc.).

The term "cycloalkyl" as used herein refers to a cyclic alkane (*i.e.*, in which a chain of carbon atoms of a hydrocarbon forms a ring), preferably including three to eight carbon atoms. Thus, exemplary cycloalkanes include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl. It should further be appreciated that cycloalkyls may also include a double bond. The term "aryl" as used herein refers to an aromatic carbon atom-containing ring, which may further include one or more non-carbon atoms (then also referred to as heteroaryl). Thus, contemplated aryl groups include cycloalkenes (*e.g.*, phenyl, naphthyl, etc.) and pyridyl. Further contemplated aryl groups may be fused (*i.e.*, covalently bound) to another aryl group, and are thus termed "fused aryl".

As also used herein, the terms "heterocycle", "cycloheteroalkyl", and "heterocyclic base" are used interchangeably herein and refer to any compound in which a plurality of atoms form a ring via a plurality of covalent bonds, wherein the ring includes at least one atom other than a carbon atom. Particularly contemplated heterocyclic bases include 5- and 6-membered rings with nitrogen, sulfur, or oxygen as the non-carbon atom (*e.g.*, imidazole, pyrrole, triazole, dihydropyr-imidine, indole, pyridine, thiazole, tetrazole etc.). Further contemplated heterocycles may be fused (*i.e.*, covalently bound) to another ring or heterocycle, and are thus termed "fused heterocycle" or "fused heterocyclic base" as used herein.

The term "alkoxy" as used herein refers to straight or branched chain alkoxides, wherein the hydrocarbon portion may have any number of carbon atoms (and may further include a double or triple bond). For example, suitable alkoxy groups include methoxy (MeO-), ethoxy, isopropoxy, etc. Similarly, the term "alkylthio" refers to straight or branched chain alkylsulfides, wherein the hydrocarbon portion may have any number of carbon atoms (and may further include a double or triple bond). For example, contemplated alkylthio groups include methylthio (MeS-),

ethylthio, isopropylthio, etc. Likewise, the term "alkylamino" refers to straight or branched alkylamines, wherein the hydrocarbon portion may have any number of carbon atoms (and may further include a double or triple bond). Furthermore, the hydrogen of the alkylamino may be substituted with another alkyl group. Therefore, exemplary alkylamino groups include methylamino, dimethylamino, ethylamino, diethylamino, isopropylamino, t-butylamino, etc.

The term "halogen" as used herein refers to fluorine, chlorine, bromine, and iodine.

It should further be recognized that all, or almost all of the above-defined groups may further be substituted with one or more substituents, which may in turn be substituted as well. For example, where a hydrogen atom in an alkyl is substituted with an amino group, one or both hydrogen atoms in the amino group may be substituted with another group (*e.g.*, alkyl or alkenyl).

The term "substituted" as used herein refers to a replacement of an atom or chemical group (*e.g.*, H, NH_2, or OH) with a functional group, and particularly contemplated functional groups include nucleophilic groups (*e.g.*, $-NH_2$, -OH, -SH, -NC, etc.), electrophilic groups (*e.g.*, C(O)OR, C(X)OH, etc.), polar groups (*e.g.*, -OH), non-polar groups (*e.g.*, aryl, alkyl, alkenyl, alkynyl, etc.), ionic groups (e.g., $-NH_3^+$), and halogens (e.g., -F, -Cl), NHCOR, $NHCONH_2$, $NHCSNH_2$, OCH_2COOH, OCH_2CONH_2, OCH_2CONHR, $OC(Me)_2COOH$, $OC(Me)_2CONH_2$, $NHCH_2COOH$, $NHCH_2CONH_2$, $NHSO_2R$, $NHSO_2CF_3$, OCH_2-heterocycles, PO_3H, SO_3H, $(CH_2)_{1-3}COOH$, CH=CHCOOH, $O(CH_2)_{1-4}COOH$, $NHCOCH_2CH(OH)COOH$, $CH(COOH)_2$, $CH(PO_3H)_2$, $OCH_2CH_2CH_2COOH$, NHCHO, and all chemically reasonable combinations thereof. Moreover, the term "substituted" also includes multiple degrees of substitution, and where multiple substituents are disclosed or claimed, the substituted compound can be independently substituted by one or more of the disclosed or claimed substituent moieties.

Thus, the term "functional group" and "substituent" are used interchangeably herein and refer to groups including nucleophilic groups (*e.g.*, $-NH_2$, -OH, -SH, -NC, -CN etc.), electrophilic groups (*e.g.*, C(O)OR, C(X)OH, C(Halogen)OR, etc.), polar groups (*e.g.*, -OH), non-polar groups (*e.g.*, aryl, alkyl, alkenyl, alkynyl, etc.), ionic groups (e.g., $-NH_3^+$), and halogens, as well as NHCOR, $NHCONH_2$, $NHCSNH_2$, OCH_2COOH, OCH_2CONH_2, OCH_2CONHR,

$OC(Me)_2COOH$, $OC(Me)_2CONH_2$, $NHCH_2COOH$, $NHCH_2CONH_2$, $NHSO_2R$, $NHSO_2CF_3$, OCH_2-heterocycles, PO_3H, SO_3H, $(CH_2)_{1-3}COOH$, $CH{=}CHCOOH$, $O(CH_2)_{1-4}COOH$, $NHCOCH_2CH(OH)COOH$, $CH(COOH)_2$, $CH(PO_3H)_2$, $OCH_2CH_2CH_2COOH$, NHCHO etc.

In one especially preferred aspect of the inventive subject matter, the inventors have discovered that numerous and chemically diverse compounds will increase fermentation of a yeast, reduce blood glucose concentration of a human, and reduce serum total and LDL-cholesterol of a human when biologically active extracts containing contemplated compounds are administered to the yeast or human. Preparation of such extracts is described in more detail in the experimental section below. Based on further observations (data not shown), the inventors specifically contemplate that such biological effects are predominantly due to activation of AMPK, or due to activation of an AMPK-associated pathway. Among compounds identified to activate AMPK, the inventors discovered that AICAR was a possible constituent of the biologically active extracts, and further contemplated that based on structural similarity and physiological relationship of AICAR to cytokinins, cytokinins may provide an active ingredient in these extracts.

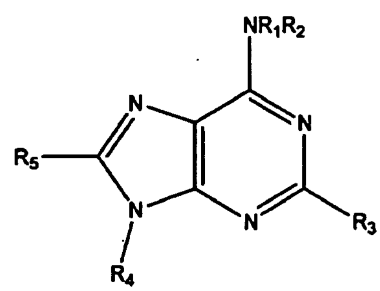
Detailed experiments (see below) with numerous other cytokinins and cytokinin analogs confirmed that (a) cytokinins and cytokinin analogs increase fermentation of a yeast, likely via a SNF1-mediated pathway, and (b) cytokinins and cytokinin analogs are likely candidates for activation of AMPK or an AMPK-associated pathway as, among other things, activation of AMPK is known to increase expression of GLUT4 (a glucose importer), to phosphorylate HMG-CoA reductase (thought to be the rate limiting enzyme in cholesterol biosynthesis) to a less active form, to reduce activity of ACC (acetyl CoA carboxylase, implicated in beta-oxidation of lipids), and to increase beta oxidation of fatty acids in selected tissues.

Therefore, the inventors generally contemplate pharmaceutical compositions, food stuffs, and nutritional supplements comprising a cytokinin or a cytokinin analog (or mixture thereof) for administration to a mammal, and especially a human in need of such products.

Contemplated Cytokinin and Cytokinin Analogs

It is generally contemplated that all compounds having cytokinin activity are suitable for use in conjunction with the teachings presented herein. Therefore, generally contemplated compounds will include naturally occurring cytokinins, as well as cytokinin analogs (which may be naturally occurring or synthetic). For example, among many other naturally occurring cytokinins, zeatin (2-Buten-1-ol, 2-methyl-4-(purin-6-ylamino)-, (E)-) and kinetin (6-(Furfurylamino)-purine) are most commonly employed in callus culture. Exemplary purine-type compounds with cytokinin activity include those described in U.S. Pat. No. 2,903,455, which is incorporated by reference herein. A person of ordinary skill in the art will readily be able to determine cytokine activity using the assay procedures described above. Exemplary synthetic (and in some case also natural) contemplated cytokinins are described in more detail below.

In one group of contemplated compounds, suitable cytokinins and cytokinin analogs will have a structure as disclosed in our co-pending provisional patent application with the docket number 22247/10600, filed August 8, 2003, which is incorporated by reference herein. Alternatively, or additionally, suitable further contemplated cytokinins and cytokinin analogs will have a structure according to Formula (I)



Formula (I)

wherein R_1 and R_2 are independently H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted alkaryl, optionally substituted heteroaryl, optionally substituted heterocycle, OH, NOH, CN, or NR_3R_4, and wherein R_3, R_4, and R_5 are independently H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl,

optionally substituted alkaryl, optionally substituted heteroaryl, optionally substituted heterocycle, NH_2, OH, NOH, CN, CF_3, O-alkyl, S-alkyl, NH-alkyl, carbohydrate radical (more preferably monosaccharide radical, and most preferably furanosyl). Alternatively, or additionally numerous suitable substituted purines are described in *Phytochemistry* 10(1), 23-8, 1971; and ibid, 7(11), 1989-94, 1968.

Still further especially contemplated purine-type cytokinin analogs include N^6-alkoximinoalkyl substituted purine compounds, and exemplary compounds having cytokinin activity and their synthesis are described in U.S. Pat. No. 5,211,738 to Sasaki et al, which is incorporated by reference herein. Alternatively, the N^6-substituent may also include a N-mono-or N-disubstituted group, and exemplary compounds with cytokinin activity and their synthesis are described in U.S. Pat. No. 5,244,487 to Oritani et al., which is also incorporated by reference herein. Where the purine substituent in the 6-position should be relatively large (and optionally distal to the heterocyclic base via a linker), adamantly or diamantyl-6-substituted purines may be employed. Various such compounds with cytokinin activity and their synthesis are described in U.S. Pat. No. 4,751,292 to Fox, which is also incorporated by reference herein. Of course it should be recognized that the purine scaffold of the exemplary compounds listed above may further be substituted as in Formula (I) above (except for the N-substituents).

In further contemplated aspects of the inventive subject matter, the inventors generally contemplate that one or more of the heteroatoms in the purine scaffold may be replaced by another heteroatom (most typically S, Se, or O), or a substituted carbon atom, wherein the substituent is defined as R_3 in Formula (I) above. Furthermore, the purine scaffold may be modified such that the five-membered ring is replaced a six-membered ring (preferably with a double bond, and most preferably with at least two conjugated double bonds). Suitable six-membered rings may include one or more heteroatoms (*e.g.*, N, S, and/or O), and additional substituents, including those listed above as R_3 in Formula (I). Thus, exemplary suitable compounds with cytokinin activity will include, for example, various pyrido[3,4-d]pyrimidine derivatives, and exemplary compounds with cytokinin activity and their synthesis are described in *Agri. Biol. Chem.*(1986), 50: 495-97, which is incorporated by reference herein. Further contemplated heterocyclic non-purine compounds with cytokinin activity are described in U.S.

Pat. No. 5,350,749 to Hackler et al., and Nishikawa, S. et al., Preparation and Structure-Activity Relationships of 4-Substituted Amino-2-methylpyrido[3,4-d]pyrimidines as Cytokinin Analogs, J. Agric. Food Chem. vol. 43, pp. 1034-1038 (1995), both of which are incorporated by reference herein.

In another group of contemplated compounds, it should be appreciated that suitable cytokinins and cytokinin analogs need not be limited to compounds having a purine scaffold or a purine analogous scaffold as exemplarily described above. Numerous compounds with cytokinin activity are known in the art that include a substituted urea or thiourea scaffold, and all of such compounds are contemplated suitable for use in conjunction with the teachings presented herein.

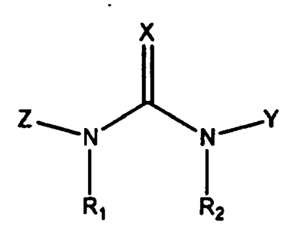
For example, 1-morpholino-3-phenylurea has been shown to have cytokinin activity in a cellular assay Bruce, Proc. Roy. Soc (London) Ser. B 165 (1966) 245-265. In another example, numerous substituted pyridyl(thio)ureas (*e.g.*, N-(2-substituted-4-pyridylureas)) have been demonstrated to have cytokinin activity as described in U.S. Pat. No. 4,279,639, to Okamoto et al., which is incorporated by reference herein. Various substituted phenyl pyridinyl ureas have been described. For example, Bruce M I, Zwar J A, Proc Roy Soc (London), Sec. B. 165 (999), 1966;245-65 disclose many N-mono- and N,N'-disubstituted ureas having cytokinin activity. N-(3,4-dichlorophenyl)-N'-3- and 4-pyridinyl ureas show such activity whereas the corresponding 2,5-dichloro compounds were inactive. In general, the authors concluded that phenyl ring substitution enhanced activity with meta substituents providing highest activity and ortho substituents lowest activity.

Similarly various substituted pyridazine ureas and thioureas have been reported to have cytokinin activity, and exemplary compounds with such activity and their synthesis is described in U.S. Pat. No. 4,331,807, to Okamoto et al., which is also incorporated by reference herein. Yet further urea-type cytokinins suitable for use in conjunction with the teachings presented herein include multi-substituted pyridinyl-phenyl ureas and thioureas (*e.g.*, N-(2,6-disubstituted 4-pyridyl)-N'-phenylurea) as described by Isogai et al. in U.S. Pat. No. 4,308,054, which is incorporated by reference herein.

Alternatively, one or both of the (hetero)aryl and/or heterocyclic substituents of the nitrogen in the urea or thiourea may be replaced by one or more iminoamine groups to form an oligo(iminoamine) with significant cytokinin activity. Exemplary oligo(iminoamine) compounds and their cytokinin activity and synthesis are described in U.S. Pat. No. 4,571,434 to Hashizume et al, which is incorporated by reference herein. On the other hand, where it is desirable to replace the oxygen or sulfur of a urea or thiourea with a nitrogen or substituted nitrogen, substituted guanidines with cytokinin activity may be obtained. For example, particularly active guanidine compounds (*e.g.*, alkyl, alkenyl, and/or alkynyl-substituted nitroguanidines) may be prepared as described in U.S. Pat. No. 4,995,903 to Lutz et al., which is incorporated by reference herein. See also: Rodaway, "Substituted nitroguanidines provide cytokinin activity during *in vitro* cultivation of plant tissues," Plant Cell Reports, 12:273-277 (1993), which is incorporated by reference herein.

In yet another group of contemplated compounds, substituted sulfonamides (*e.g.*, O-sulfamylalkylbenzenesulfonamides) may be employed in conjunction with the teachings presented herein, and especially preferred sulfonamide compounds include those described by Sauers in U.S. Pat. No. 4,397,679, which is incorporated by reference herein. Further contemplated compounds also include various substituted ethanolamines with cytokinin activity, and especially those that include at least one aromatic group coupled to the amino group. For example, suitable N-dialkyl-alkaryl-substituted ethanolamines are described in U.S. Pat. No. 4,929,267 to Suzuki et al., which is incorporated by reference herein.

Thus, and viewed from another perspective, suitable non-purine compounds with cytokinin activity may have a general structure according to Formula (II)



Formula (II)

in which X is O, S, or NR_3; Y and Z are independently H, a polar group, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted alkaryl, optionally substituted heteroaryl, optionally substituted heterocycle; R_1 and R_2 are independently H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted alkaryl, optionally substituted heteroaryl, optionally substituted heterocycle, OH, NOH, CN, or NR_3R_4, and wherein R_3 and R_4 are independently H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted alkaryl, optionally substituted heteroaryl, optionally substituted heterocycle, NH_2, OH, NOH, CN, CF_3, O-alkyl, S-alkyl, or NH-alkyl.

In a still further contemplated group of suitable compounds, non-homogenous preparations of mycelia of and growth medium for various basidiomycetes have shown significant cytokinin activity, and exemplary preparations and activities are described in U.S. Pat. No. 4,281,021 to Iizuka et al., which is incorporated by reference herein. Moreover, the inventors also specifically contemplate anticytokinins for use herein, and exemplary anticytokinins are described in U.S. Pat. No. 3,988,338 to Skoog et al.

Compositions Comprising Contemplated Compounds

Of course it should be recognized that use of contemplated compounds is not limited to *in vitro* systems, and it is particularly contemplated that suitable compounds will be formulated for administration to a mammal, and especially to a human with a condition that is responsive to the administration of such compounds. Therefore, where contemplated compounds are administered in a pharmacological composition, it is contemplated that contemplated compounds can be formulated in admixture with a pharmaceutically acceptable carrier. For example, contemplated compounds can be administered orally as pharmacologically acceptable salts, or intravenously in a physiological saline solution (*e.g.*, buffered to a pH of about 7.2 to 7.5). Conventional buffers such as phosphates, bicarbonates or citrates can be used for this purpose. Of course, one of ordinary skill in the art may modify the formulations within the teachings of the specification to provide numerous formulations for a particular route of administration. In particular, contemplated compounds may be modified to render them more soluble in water or other vehicle,

which for example, may be easily accomplished with minor modifications (salt formulation, esterification, *etc.*) that are well within the ordinary skill in the art. It is also well within the ordinary skill of the art to modify the route of administration and dosage regimen of a particular compound in order to manage the pharmacokinetics of the present compounds for maximum beneficial effect in a patient.

In certain pharmaceutical dosage forms, prodrug forms of contemplated compounds may be formed for various purposes, including reduction of toxicity, increasing the organ or target cell specificity, etc. Among various prodrug forms, acylated (acetylated or other) derivatives, pyridine esters and various salt forms of the present compounds are preferred. One of ordinary skill in the art will recognize how to readily modify the present compounds to prodrug forms to facilitate delivery of active compounds to a target site within the host organism or patient. One of ordinary skill in the art will also take advantage of favorable pharmacokinetic parameters of the prodrug forms, where applicable, in delivering the present compounds to a targeted site within the host organism or patient to maximize the intended effect of the compound. Similarly, it should be appreciated that contemplated compounds may also be metabolized to their biologically active form (*e.g.*, via hydroxylation, glycolsylation, oxidation etc.), and all metabolites of the compounds herein are therefore specifically contemplated.

In addition, contemplated compounds may be administered alone or in combination with other agents for the treatment of various diseases or conditions. Combination therapies according to the present invention comprise the administration of at least one compound of the present invention or a functional derivative thereof and at least one other pharmaceutically active ingredient. Preferred second pharmaceutically active agents for combination therapy include anti-dyslipidemic drugs (*e.g.*, statins), antidiabetic drugs (*e.g.*, metformin), immune modulators (*e.g.*, cytokines, interferons, histidine, etc.), and/or anti-inflammatory compounds (*e.g.*, steroids). The active ingredient(s) and pharmaceutically active agents may be administered separately or together and when administered separately this may occur simultaneously or separately in any order. Furthermore, the amounts of the active ingredient(s) and pharmaceutically active agent(s) and the relative timings of administration will be selected in order to achieve the desired combined therapeutic effect.

Alternatively, contemplated compounds may also be administered as nutritional supplements or food stuff. For example, contemplated compounds may be provided as an extract of a plant (*e.g.*, from malted barley), or isolated to a relatively high degree of purity (*e.g.*, at least 50 wt%, more typically at least 70 wt%, and even more typically at least 85 wt%) from a plant. Such extracts are preferably prepared in solid form (*e.g.*, in a tablet or capsule), but may also be in a liquid preparation. Moreover, purified or synthetic cytokinins or cytokinin analogs may be included into a snack bar, breakfast cereal, drink, etc.

Indications for Use of Contemplated Cytokinins and Cytokinin Analogs

It is generally contemplated that all diseases or conditions associated with activation of AMPK or activation of an AMPK-associated pathway can the treated using contemplated compounds. For example, numerous diseases and conditions would benefit from an activation of AMPK in a patient, and the following list provides exemplary guidance on contemplated indications for use of the compounds presented herein.

Dyslipidemia

Hepatic acetyl-CoA carboxylase (ACC) and 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) are two targets for the AMPK system, catalyzing the key regulatory steps in fatty acid and sterol synthesis, respectively (Winder et al, Am J Physiol, 2777: E1-10, 1999, the entirety of which is herein incorporated by reference.) Activation of AMPK serves to inhibit both these lipid biosynthetic pathways, as well as triglyceride synthesis. Moreover, it is contemplated that activated AMPK inhibits the L-type pyruvate kinase and fatty acid synthase gene expression.

Reduction of activity of ACC in the liver cell also leads to decreases in the concentration of the product of ACC, *i.e.*, malonyl-CoA, which has marked effects on fatty acid oxidation. Malonyl-CoA is a potent inhibitor of carnitine palmitoyltransferase-1 (CPT-1), the "gatekeeper" for entry of fatty acids into the mitochondria. In the liver, fatty acid oxidation can be considered to be an essential component of the pathway for synthesis of ketone bodies: increases in fatty acid oxidation lead to increased hepatic ketogenesis. It is therefore contemplated that administration of contemplated compounds at a concentration effective to activate AMPK in the liver would result in decreases in fatty acid, triglyceride, and sterol synthesis and increases in

fatty acid oxidation and ketogenesis. The term "dyslipidemia" as used herein refers to a clinical abnormality (*e.g.*, elevation above threshold considered upper limit for healthy individual) in at least one blood lipid or component with lipid associated thereto, including triglycerides, fatty acids, and cholesterol (HDL-, LDL-, VLDL, etc.).

Viewed from another perspective, treatment with contemplated compounds to increase AMPK activity will useful in reducing fatty acid synthesis, sterol synthesis, triglyceride synthesis and fatty acid synthase gene expression, and in ameliorating disorders that are characterized by elevations in one or more of these lipids, or that are exacerbated by the effects of one or more of these lipids. Of additional benefit is also the AMPK-mediated increase in activity in fatty acid oxidation and ketogenesis, where increased ketogenesis is desired.

Obesity

Hormone-sensitive lipase (HSL) is a target for AMPK in adipose tissue. Activation of AMPK has been shown to inhibit lipogenesis by phosphorylation of ACC and also to inhibit isoprenaline-stimulated lipolysis. Thus, treatment with contemplated compounds to increase AMPK activity is thought to inhibiting lipogenesis and to increase isoprenaline-stimulated lipolysis.

Furthermore, 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), has been found to inhibit the differentiation of 3T3-L1 adipocytes, if added at an early phase of differentiation. (*Biochem Biophys Res Commun*, 286(5):852-6, which is incorporated herein by reference). AICAR blocks the expression of the late adipogenic markers, fatty acid synthase and acetyl-CoA carboxylase, and of the transcription factors, C/EBPalpha and PPARgamma. AICAR further inhibits early clonal expansion of pre-adipocytes, appears to prevent C/EBPbeta expression during the intermediate stage of differentiation, and inhibits the late phase expression of CHOP-10, an antagonist of C/EBPbeta. Thus, given the inhibitory role for AMPK in the process of adipose differentiation, treatment with contemplated compounds to increase AMPK activity will likely inhibit adipogenesis.

Insulin Oversecretion

It is generally accepted in the art that activated AMPK inhibits insulin secretion, and as contemplated compounds were demonstrated to activate AMPK, it should be recognized that treatment with such compounds should provide a significant reduction in insulin secretion. Consequently, conditions associated with oversecretion of insulin may be treated using contemplated compounds.

Insufficient Glucose Uptake in Muscle Cells

It has been observed that exercise and/or electrical stimulation of various muscles increases AMPK activity, and consequently increases glucose uptake. It has also been observed that glucose uptake is increased by chemical activation of AMPK with AICA-riboside. Based on these observations, it has been hypothesized that muscle contraction plays a role in stimulating glucose uptake in muscle, where one mechanism underlying increased uptake stems from activated AMPK increasing GLUT-4 translocation from microvesicles to sarcolemmal membranes in muscle. Thus, treatment with contemplated compounds to increase AMPK activity is though to be beneficial in enhancing glucose uptake into muscle cells, and in ameliorating disorders that are characterized by decreased glucose uptake in muscle cells, or that are exacerbated by the effects of decreased glucose uptake in muscle cells.

Modulation of Stability of Selected mRNA species

HuR is an RNA binding protein that functions to stabilize a variety of target mRNA transcripts, including those encoding p21, cyclinA and cyclinB1. It has been shown that the presence of activated AMPK results in reduced levels of cytoplasmic HuR, and in turn, in reduced concentrations and half-lives of mRNA encoding p21, cyclinA and cyclinB1 (see *e.g.*, *Mol Cell Biol*, 22(10):345-36, 20002, which is incorporated herein by reference). Thus, treatment with contemplated compounds will increase AMPK activity, and thus reduce levels of cytoplasmic HuR, which is though to reduce concentrations/half-lives of a variety of target mRNA transcripts, including those ending p21, cyclinA and cyclinB1.

Premature Apoptosis

Activated AMPK has been shown to provide protection against glucocorticoid-induced apoptosis and to restore cell viability and inhibit DNA laddering in dexamethasone-treated thymocytes (see *e.g.*, Biochem Biophys Res Commun, 243(3):821-6, 1998, which is incorporated herein by reference). Furthermore, activated AMPK has been shown to provide protection against dexamethasone-induced activation of caspase 3-like enzymes, which are believed to play a pivotal role in apoptotic cell death. Thus, treatment with contemplated compounds to increase AMPK activity may provide protection against glucocorticoid-induced apoptosis.

Ischemia

Conditions and disorders associated with AMPK regulation of cellular responses to stresses, including ischemia, are among those treatable by administering a composition comprising a compound that activates AMPK. In several non-vascular tissues, AMPK appears to modulate the cellular response to stresses such as ischemia. In liver and muscle, AMPK phosphorylates and inhibits acetyl CoA carboxylase (ACC), leading to an increase in fatty acid oxidation; in muscle, AMPK activation is associated with an increase in glucose transport. Furthermore, incubation of human umbilical vein endothelial cells (HUVEC) with an AMPK activator has been shown to cause a 5-fold activation of AMPK, which was accompanied by a 70% decrease in ACC activity and a 2-fold increase in fatty acid oxidation. (*Biochem Biophys Res Commun*, 265(1):112-5, 1999, which is incorporated herein by reference). However, in this same study, glucose uptake and glycolysis, the dominant energy-producing pathway in HUVEC, were diminished by 40-60% under these conditions. Despite this, cellular ATP levels were increased by 35%. Thus, treatment with contemplated compounds to increase AMPK activity is expected to result in major alterations in endothelial cell energy balance, which are useful in providing protection against cellular stresses in conditions including ischemia.

Metabolic and Excitotoxic Insults

It is well known in the art that the brain has a high metabolic rate and is relatively sensitive to changes in the supply of glucose and oxygen. The expression of AMPK in embryonic and adult brain and its role in modifying neuronal survival under conditions of cellular stress

have been investigated (*J Mol Neurosci*, 17(1): 45-58, 2001). Catalytic (alpha 1 and alpha 2) and noncatalytic (beta 2 and gamma 1) subunits of AMPK are present at high levels in embryonic hippocampal neurons *in vivo* and in cell culture. In the adult brain, the catalytic subunits alpha 1 and alpha 2 are present in neurons throughout the brain. The AMPK-activating agent AICAR protected hippocampal neurons against death induced by glucose deprivation, chemical hypoxia, and exposure to glutamate and amyloid beta-peptide. Suppression of levels of the AMPK alpha 1 and alpha 2 subunits using antisense technology resulted in enhanced neuronal death following glucose deprivation, and abolished the neuroprotective effect of AICAR. Thus, given the role of AMPK activation in modifying neuronal survival under conditions of cellular stress, treatment with contemplated compounds to increase AMPK activity is thought to provide protection of neurons against metabolic and excitotoxic insults.

Similarly, conditions and disorders associated with hypoxia may be treated using contemplated compounds. AMPK is believed to play a role in regulating ketone body production by astrocytes. (*J Neurochem*, 73(4): 1674-82, 1999). Incubation of astrocytes with AICAR has been shown to stimulate both ketogenesis from palmitate and carnitine palmitoyltransferase I concomitant to a decrease of intracellular malonyl-CoA levels and an inhibition of acetyl-CoA carboxylase/fatty acid synthesis and 3-hydroxy-3-methylglutaryl-CoA reductase/cholesterol synthesis. Moreover, microdialysis experiments have shown AICAR to stimulate brain ketogenesis markedly. Incubation of astrocytes with azide has been shown to lead to a remarkable drop of fatty acid beta-oxidation. However, activation of AMPK during hypoxia was shown to compensate the depression of beta-oxidation, thereby sustaining ketone body production. The effect is believed to rely on the following cascade: hypoxia leads to an increase of the AMP/ATP ratio, which triggers AMPK stimulation, which in turn results in acetyl-CoA carboxylase inhibition. Consequently, malonyl-CoA concentration decreasesm and carnitine palmitoyltransferase I is activated, thus enhancing ketogenesis. Furthermore, incubation of neurons with azide has been shown to blunt lactate oxidation, but not 3-hydroxybutyrate oxidation. Thus, given the role of AMPK activation in regulating ketone body production by astrocytes, treatment with contemplated compounds to increase AMPK activity is useful in promoting astrocytes to produce ketone bodies as a substrate for neuronal oxidative metabolism during hypoxia.

Reduced Insulin Sensitivity

Conditions and disorders associated with diminished insulin sensitivity of muscle glucose transport may be treated by administration of contemplated compounds. A study to determine the degree to which hypoxia and AICAR also induce an increase in insulin sensitivity has been reported (*Am J Physiol Endocrinol Metab*, 282(1): E18-23, 2002). This study found that the increase in glucose transport in response to 30 microU/ml insulin was about two-fold greater in rat epitrochlearis muscles that had been made hypoxic or treated with AICAR 3.5 h previously than in untreated control muscles. This increase in insulin sensitivity was similar to that induced by a 2-h bout of swimming or 10 min of *in vitro* electrically stimulated contractions. Neither phosphatidylinositol 3-kinase activity nor protein kinase B (PKB) phosphorylation in response to 30 microU/ml insulin was enhanced by prior exercise or AICAR treatment that increased insulin sensitivity of glucose transport.

Inhibition of protein synthesis by inclusion of cycloheximide in the incubation medium for 3.5 h after exercise did not prevent the increase in insulin sensitivity. Contractions, hypoxia, and treatment with AICAR all caused a two- to three-fold increase in AMPK activity over the resting level. These results provide evidence that the increase in insulin sensitivity of muscle glucose transport that follows exercise is mediated by activation of AMPK. Thus, treatment with contemplated compounds is thought to provide increased insulin sensitivity of muscle glucose transport.

Hepatic ischemia-reperfusion

Hepatic ischemia-reperfusion (I/R) injury associated with liver transplantation and hepatic resections may be reduced by administering a composition comprising a compound that activates AMPK. Preconditioning is known to preserve energy metabolism in liver during sustained ischemia. A study has been reported that investigates: 1) whether preconditioning induces AMPK activation; and 2) if AMPK activation leads to ATP preservation and reduced lactate accumulation during prolonged ischemia and its relationship with NO (*Hepatology*, 34(6): 1164-73, 2001). Preconditioning was reported to activate AMPK and concomitantly reduce ATP degradation, lactate accumulation, and hepatic injury. The administration of an AMPK activator,

AICAR, before ischemia simulated the benefits of preconditioning on energy metabolism and hepatic injury. The inhibition of AMPK abolished the protective effects of preconditioning. The effect of AMPK on energy metabolism was independent of NO because the inhibition of NO synthesis in the preconditioned group and the administration of the NO donor before ischemia, or to the preconditioned group with previous inhibition of AMPK, had no effect on energy metabolism. Thus, given the role of AMPK activation in the protective effect against ischemia, treatment with contemplated compounds to increase AMPK activity is contemplated for surgical and pharmacological strategies aimed at reducing hepatic I/R injury.

Hyperglycemia

It has recently been reported that therapeutic doses of metformin increase AMPK activity *in vivo* in subjects with type 2 diabetes (*Diabetes*, 51(7): 2074-81, 2002). Metformin treatment for 10 weeks significantly increased AMPK alpha 2 activity in the skeletal muscle, and this was associated with increased phosphorylation of AMPK on Thr172 and decreased acetyl-CoA carboxylase-2 activity. The increase in AMPK alpha 2 activity was likely due to a change in muscle energy status because ATP and phosphocreatine concentrations were lower after metformin treatment. Metformin-induced increases in AMPK activity were associated with higher rates of glucose disposal and muscle glycogen concentrations. These findings suggest that the metabolic effects of metformin in subjects with type 2 diabetes may be mediated by the activation of AMPK alpha 2. Given the hypoglycemic effect imparted by the activation of AMPK, treatment with contemplated compounds to increase AMPK activity may be useful to lower blood glucose concentrations by decreasing hepatic glucose production and increasing glucose disposal in skeletal muscle.

Insulin Resistance Syndrome

Insulin resistance syndrome is associated with obesity, type 2 diabetes, and muscle paralysis (see *e.g.*, WO 01/97816 A1 and WO 01/93874 A1). Insulin resistance syndrome is also associated with several risk factors for cardiovascular disease. Chronic chemical activation of AMP-activated protein kinase by the adenosine analog AICAR has been shown to augment insulin action, upregulate mitochondrial enzymes in skeletal muscles, and decrease the content of

intra-abdominal fat, including that occurring in obesity. Furthermore, acute AICAR exposure has been found to reduce sterol and fatty acid synthesis in rat hepatocytes incubated *in vitro* as well as suppress endogenous glucose production in rats under euglycemic clamp conditions. A recent study investigated whether chronic AICAR administration, in addition to the beneficial effects on insulin sensitivity in type 2 diabetes, is capable of improving other phenotypes associated with the insulin resistance syndrome (*Diabetes*, 51(7): 2199-206, 2002). AICAR administration significantly reduced plasma triglyceride levels ($P < 0.01$ for AICAR vs. AL, and $P = 0.05$ for AICAR vs. PF) and free fatty acids ($P < 0.01$ for AICAR vs. AL, and $P < 0.05$ for AICAR vs. PF) and increased HDL cholesterol levels ($P < 0.01$ for AICAR vs. AL and PF). AICAR treatment also lowered systolic blood pressure by 14.6 +/- 4.3 mmHg ($P < 0.05$), and AICAR-treated animals exhibited a tendency toward decreased intra-abdominal fat content.

Furthermore, AICAR administration normalized the oral glucose tolerance test and decreased fasting concentrations of glucose and insulin close to the level of the lean animals. Finally, in line with previous findings, AICAR treatment was also found to enhance GLUT4 protein expression and to increase maximally insulin-stimulated glucose transport in primarily white fast-twitch muscles. In view of the strong evidence that activating AMPK improves glucose tolerance, improves the lipid profile, and reduces systolic blood pressure, treatment with contemplated compounds to increase AMPK activity is useful to reduce metabolic disturbances and lowers blood pressure characteristic of insulin resistance syndrome.

Inflammation and Immune Modulation

It has previously been demonstrated that activation of AMPK inhibits NFkB activity in endothelial cells (*Biochem. Soc. Trans.* (2001) 31, (202–206)). Consequently, it should be recognized that numerous processes involving NFkB controlled expression may be affected by administration of contemplated compounds, and it is particularly contemplated that such compounds may be employed as anti-inflammatory agent. Furthermore, it is recognized that activation of AMP-activated protein kinase stimulates nitric-oxide synthesis in human aortic endothelial cells (see *e.g.*, *J. Biol. Chem.*, August 22, 2003; 278(34): 31629 - 31639). Consequently, numerous processes associated with nitric oxide may be controlled by administration of contemplated compounds.

Use of Anticytokinins

In another example, numerous diseases and conditions would benefit from a reduction in activation of AMPK in a patient, and the following list provides exemplary guidance on contemplated indications for use of the compounds presented herein.

Neoplasms

It is well established that nutrient deprivation activates AMPK (*supra*), and that tumors in a relatively early stage are dependent on nutrient diffusion. Thus, when a tumor reaches a critical mass, AMPK will be activated in at least some cells due to lack of glucose and other growth factors. Consequently, the inventors contemplate that contemplated anticytokinins may be employed to block energy salvage pathways of tumor cells (see *e.g.*, Oncogene. 2002 Sep 5;21(39):6082-90: Critical roles of AMP-activated protein kinase in constitutive tolerance of cancer cells to nutrient deprivation and tumor formation by Kato et al.).

Ventricular Tachyarrhythmias

Recent studies have shown that ventricular tachyarrhythmias frequently arise as a consequence of activated AMPK. Consequently, it is contemplated that compounds according to the inventive subject matter with anti-cytokinins activity may be employed as treatment modalities against ventricular tachyarrhythmias.

Examples

Effect of Selected Compounds on Glut-4, activated AMPK, and activated Akt

The levels of Glut-4, activated AMPK and activated Akt were measured in mouse muscle cells C2C12 (from ATTC) and in primary culture of human skeletal muscle cells (Clonetics, Inc.) using Western immunoblotting. C2C12 cells were plated at 1.5x1-exp5 cells per mL/well (12-well plate) in standard cell culture medium (DMEM supplemented with 10% fetal bovine serum (FBS), 25mM glucose, 20mM Hepes, 4mM glutamine and 2 mM sodium pyruvate. 48 hrs after the plating, medium was changed to differentiation medium (DMEM supplemented with 5 mM of glucose and 0.5% of FBS) for next 3-4 days to stimulate the formation of myotubes. Three hrs

before the treatment with selected agents, cells were washed with PBS and transferred to PBS supplemented with 5mM of glucose.

Human skeletal muscle cells (HSKM) were cultured in SKBM-2 mediums provided by Clonetics. 48 hrs after cell plating, medium was changed to SKBM medium to stimulate differentiation of the cells to myotubes. When differentiated, the myotubes were transferred to PBS supplemented with 5mM glucose for three hrs before the treatment.

Analysis of C2C12 cells for the level of activated AMPK, Akt and the level of GLUT-4 was performed in the same experimental system. The cells were treated for 30 minutes at 37 °C. After the treatment, the cells were washed with ice-cold PBS and lysed with 80ul of lysis buffer/well (M-PER buffer from Pierce supplemented with protease and phosphatase inhibitor mix (Calbiochem) for 10 minutes on ice. Next, the plates were transferred to –20 °C to improve the lysis of the cells. Next cells were sonicated for 5 minutes and lysate was transferred to Eppendorf tubes and centrifuged at 14,000rpm for 10 minutes. Supernatants were collected in fresh Eppendorf tubes and kept on ice to measure the amount of total proteins. 3μl of each lysate was used to measure the protein concentration using standard Bradford method (Biorad). Subsequently, 20μg per sample of sample protein was used for Western analysis using NuPage 10% Bis/Tris gels (Invitrogen). After exposure of membranes to primary and secondary antibodies AMPK, AKT or Glut-4 was detected using ECL-Plus (Amersham) following producer's instruction. Chemilumiscent signals were detected by using ChemiDoc system from Biorad. Intensity of detected signals were analyzed and measured using Quantity One software (Biorad). Alternatively, the level of phosphorylated AMPK was detected using ECL kit from Amersham and short exposure to Kodak films.

Experimental setup: Cell Culture was followed by treatment with selected contemplated compounds, which was followed by cell lysis and western blot analysis for AMPK, Akt, GLUT4, total AMPK, and total Akt. Signals were acquired accordingly. Primary antibodies used in these studies are the following: Anti-phospho-AMPK (Thr172), mouse, rabbit IgG, from Cell Signaling, #2531; Anti-phospho-Akt (Ser473), mouse, rabbit IgG, Cell Signaling, #9271; Anti-Glut-4, mouse, rabbit IgG, Calbiochem, #400064; Anti-AMPK (total), mouse, rabbit IgG, Cell Signaling, #2532; Anti-Akt (total), mouse, rabbit IgG, Cell Signaling, #9272.

Results for AMPK Activation

The effects of various cytokinins and cytokinin analogs (some data not shown) on AMPK activity are summarized in the table below. The results demonstrate that most of the tested agents significantly stimulate AMPK activity, with some resulting in over 10 fold increases in activity compared the untreated control. The more potent compounds include derivatives of adenine, cytidine and guanosine as well as kinetin and zeatin. The table refers to multiple independent experiments where multiple identical concentrations for the same reagents are indicated.

AGENT	CONCENTRATION (microM)	FOLD AMPK ACTIVATION (OVER CONTROL)
Adenosine	12.5	1.83
	5.0	1.64
	2.5	1.87
N^6-Acetyl-Adenosine	12.5	2.18
	5.0	2.41
	2.5	1.08
Benzyl-Adenine	50.0	2.92
	5.0	2.70
	0.5	2.18
Gamma, Gamma-Dimethylally-6-Aminopurine	50.0	2.11
	5.0	2.45
	0.5	2.82
Dihydro-Zeatin	50.0	0.88
	5.0	0.59
	0.5	2.25
Zeatin	1.0	2.33
	10.0	2.04
	1.0	2.15
Trans-Zeatin	10.0	4.27
	1.0	2.33
Guanosine	5.0	3.70
N^2-Acetyl-Guanosine	2.0	2.20
	0.8	2.20
	0.3	3.75
	1.5	4.28
	7.5	1.71
	37.5	2.28
N^2-Acetyl-Guanine	0.3	5.42
	1.5	5.85
	7.5	6.00
	37.5	6.51

Kinetin	0.8 0.8 2.0 10.0 0.1 0.3 1.0 3.0	3.60 2.40 2.70 12.80 5.30 8.12 19.50 14.50
Kinetin Riboside	3.0	12.00
Metformin	2.0 milliM	1.42
Rosiglitazone	3.0	3.50

Results for Akt Activity

The effects of various cytokinins and cytokinin analogs on Akt activity are summarized in the table below. Interestingly, many of the potent AMPK stimulators had only marginal effect on Akt activity. For example, zeatin is a potent stimulator of AMPK but not Akt. However, guanosine, N^2-Acetyl-Guanosine and N^2-Acetyl-Guanine were observed to be potent activators of AMPK as well as Akt. The table refers to multiple independent experiments where multiple identical concentrations for the same reagents are indicated.

AGENT	CONCENTRATION (microM)	FOLD AKT ACTIVATION (OVER CONTROL)
Kinetin	5.0 2.0 0.8 8.1 2.7 0.9 0.3	2.07 3.35 3.17 0.24 3.21 3.81 5.08
Kinetin Riboside	5.0 2.0 0.8	3.32 5.14 3.71
Zeatin	10.0 1.0	1.36 0.95
Trans-Zeatin	10.0 1.0	0.86 0.90
Gamma, Gamma-Dimethylally-6-Aminopurine	2.0 0.8 0.8	1.32 1.90 3.56

N^4-Acetyl-Cytidine	5.0	1.64
	2.0	1.46
	0.8	2.45
	5.0	1.36
	2.0	1.50
N^2-Acetyl-Guanosine	5.0	1.23
	0.8	1.75
	0.3	1.92
	0.1	2.57
	2.0	2.17
	0.8	2.95
	7.5	1.68
	1.5	1.55
	0.3	2.57
N^2-Acetyl-Guanine	7.5	2.58
	1.5	3.58
	0.3	3.50
	7.5	1.95
	1.5	1.64
	0.3	2.44
AICAR	500.0	5.45
	50.0	2.20
Metformin	20.0 milliM	2.32
	2.0 milliM	2.70
Insulin	0.10 nanoM	1.75
	50.0 nanoM	3.28
	25.0 nanoM	3.40
Rosiglitazone	27.0	0.71
	9.0	1.78
	3.0	2.34
	3.0	5.01
	1.0	2.83

Results for GLUT-4

The effects of kinetin, N^2-Acetyl-Guanosine and N^2-Acetyl-Guanine on GLUT-4 protein level in C2C12 cells were investigated following the same experimental design as described for AMPK and AKT. Anti-Glut-4 antibody used in this study was from Calbiochem. The results summarized in the table below demonstrate that kinetin, N^2-Acetyl-Guanosine and N^2-Acetyl-Guanine potently increase GLUT-4 protein level in C2C12 cells at different range and in a dose-dependent manner. The table refers to multiple independent experiments where multiple identical concentrations for the same reagents are indicated.

AGENT	CONCENTRATION (microM)	FOLD CHANGE IN GLUT-4 LEVEL (OVER CONTROL)
Rosiglitazone	3.0 9.0 27.0 3.0 3.0 3.0	3.82 3.61 3.19 2.13 4.37 2.98
Metformin	2000	1.50
Kinetin	0.3 0.9 2.7 8.1 0.8 0.3 0.8 2.0	3.45 4.00 3.88 1.11 3.46 3.95 2.36 1.88
N^2-Acetyl-Guanine	0.3 1.5 7.5 37.5	3.94 3.84 3.24 2.80
N^2-Acetyl-Guanosine	0.3 1.5 7.5 37.5	1.21 1.74 3.14 3.03

Glucose Uptake

Total glucose uptake was measured using fluorescent glucose analog from Molecular Probes. Briefly, muscle cells were treated with kinetin, N^2-Acetyl-Guanosine and N^2-Acetyl-Guanine for 30 minutes at 37C first and subsequently, these cells were exposed to 500 μM of fluorescent glucose analog for 5 minutes at room temperature. Next, cells were washed twice with cold Krebs-Hepes buffered solution and fixed in 70% ethanol in water. Fluorescence of fluorescent glucose in the cells was measured using fluorescent plate reader at 480/530 nm (excitation/emission). The results summarized in the table below demonstrate that kinetin, N^2-Acetyl-Guanosine and N^2-Acetyl-Guanine each potently enhance glucose uptake in muscle cells *in vitro*. The table refers to multiple independent experiments where multiple identical concentrations for the same reagents are indicated.

AGENT	CONCENTRATION	AVERAGE (N=3)	FOLD CHANGE IN TOTAL GLUCOSE UPTAKE (OVER CONTROL)
N^2-Acetyl-Guanosine	0.0	20.3 +/-0.1	-
	0.3	44.1 +/-0.7	2.17
	1.5	54,3 +/-0.9	2.67
	7.5	61.7 +/-1.3	3.03
	0.00	46.5 +/- 1.2	-
	0.15	90.5 +/- 1.7	1.94
	0.75	109.5 +/-2.6	2.35
	3.75	148.7 +/- 8.5	3.18
N^2-Acetyl-Guanine	0.3	54.5 +/- 1.7	2.68
	1.5	55.2 +/- 0.8	2.71
	7.5	59.6 +/-0.4	2.93
	0.00	46.5 +/-1.2	-
	0.15	86.4+/- 2.3	1.85
	3.75	115.9+/- 3.7	2.48
Kinetin	0.00	47+/- 0.7	-
	0.15	88.6+/- 0.9	1.88
	0.75	103.3+/-2.1	2.19
	3.75	102.6+/-4.7	2.18
	0.0	28.9+/-0.1	-
	0.3	86.0+/-0.7	2.97
	1.5	110.6+/-2.3	3.82
	7.5	56.6+/-1.4	1.95
Rosiglitazone	3.0	47.3 +/-1.1	2.33
	30.0	56.5 +/- 1.4	2.78
	0.0	52.1 +/-0.2	-
	3.0	122.4 +/-3.7	2.34

Increase in Fermentation

The ability of a compound to increase the fermentation rate of yeast was evaluated as a potential initial screen for selected compounds having a desired biological activity. The fermentation rate of *Saccharomyces cerevisiae* was measured according to Warburg methodology by determination of evolved carbon dioxide during fermentation process.

Dry commercial Baker's Yeast used in all experiments. The fermentation medium comprised about 2.5% glucose in 63 mM phosphate buffer (pH 6.3). The effects of test substances on the rate yeast fermentation were compared with the rate yeast fermentation in control tests lacking the test substance. Tests were conducted employing an apparatus comprising two parallel units for simultaneous fermentation rate measurement in presence of the tested substance (Probe) and in absence of it (Control). Units were identical consisting of the fermentation vessels (Erlenmeyer flask, 100 ml) connected to a manometric U tube (h =25 cm,

diameter = 2 mm) equipped with valve and filled with light liquid such as isopropanol. Erlenmeyer flasks were equipped with magnetic stirring bars and placed on a magnetic stirrer (20-25 cm diameter). Generally, the amount of yeast employed was fixed at about 50 mg, while the volume of fermentation medium was generally about 20 mL. The amounts of tested substances varied from 0.020 mg to 0.700 mg, depending on their activities.

Both systems were simultaneously pre-incubated under aerobic conditions for 10 minutes (open valves). Fermentation process under anaerobic conditions and the real rate measurements started after closing the valves. The fermentation rates were monitored 5 minute interval for 50 minutes by observing the differences of the liquid heights in U-tubes (which differences correspond to a pressure difference between a reaction vessel and an atmosphere - deltaP). The results are summarized in the table below and clearly demonstrate that various of the contemplated compounds, including cis-zeatin, trans-zeatin, dihydro-zeatin, benzyl-adenine, gamma,gamma-dimethylallyl-6-aminopurine, kinetin riboside, N^6-acetyl-adenosine, N^2-acetyl-Guanosine, N^4-acetyl-cytidine, enhance yeast fermentation rates.

AGENT	CONCENTRATION (microM)	ENHANCEMENT OF YEAST FERMENTATION RATE (AFTER 60 MINUTES; CONTROL=1)
Cis-Zeatin	6.2	1.44
Trans-Zeatin	8.9	1.17
Dihydro-Zeatin	9.1	1.738
Benzyl-Adenine	11.3	1.74
Gamma,gamma-dimethylallyl-6-aminopurine	21.3	1.92
Kinetin Riboside	101	1.67
N6-acetyl-Adenosine	30.7	1.92
N2-acetyl-Guanosine	13.3	1.98
N4-acetyl-Cytidine	15.1	2.05
AICAR	135.5	1.77

Liver Metabolism and Diseases

It has been reported that HNF4 (hepatic nuclear factor alpha 4) plays a crucial role in energy metabolism in liver, presumably by virtue of HNF4 being a downstream target in a

AMPK-associated pathway in which AMPK-activated protein kinase regulates HNF4alpha transcriptional activity (see *e.g.*, Hong YH et al, J. Biol Chem, 273(30) 27495-501, 2003). Thus, compounds according to the inventive subject matter may be employed in regulating energy metabolism in the liver.

Furthermore, in a presumably AMPK-independent manner, selected cytokinins were shown to have inhibitory effects on xanthine oxidase (see *e.g.*, Sheu SY et al, *Anticancer Res*, 16(1), 311-5, 1996). Xanthine oxidase is frequently increased in hepatitis, and according to Sheu et al., selected zeatins may be used to decrease the activity of this enzyme as a form of treatment in hepatitis.

Viral Infections

Based on the relatively wide range of biological responses to administration of cytokinins in virus-infected plants, and the observation that various intracellular mechanisms that may confer antiviral effects appear to be conserved between plants and mammals, the inventors contemplate that administration of cytokinins to a mammal infected with a virus may indeed provide at least some antiviral effect. Exemplary antiviral mechanisms (which may be conserved) are provided below: Endogenous changes in cytokinin activity in systemically virus-infected plants by Pennazio S, et al, *New Microbiol*, 21(4), 419-426, 1999. Expression of the gene for small GTP binding protein in transgenic tobacco elevates endogenous cytokinin level, abnormally induces salicylic acid in response to wounding, and increases resistance to tobacco mosaic virus infection, by Sano H et al, *PNAS* 91(22), 10556-60, 2001. Cytokinins in pathogenesis and disease resistance of Pyrenophera teres-barley and Dreschslera maydis-maize interaction during early stages of infection, by Angra-Sharma R, et al, *Mycopathologia,* 148(2), 87-95, 2001, and Infection of soybean and pea nodules by Rhizobium spp purine auxotrophs in the presence of AICAR, by Newman JD et al, *J Bacteriol* 176(11), 3286-94, 1994

Consequently, the inventors contemplate pharmaceutical compositions comprising at least one cytokinin or cytokinin analog at a concentration effective to activate AMPK or to activate an AMPK-associated pathway in a mammal when the composition is administered to the mammal, wherein particularly contemplated pharmaceutical compositions will include one or

more cytokinin or cytokinin analog as contemplated above. In even more preferred aspects of the inventive subject matter, it is contemplated that administration of such pharmaceutical compositions will be indicated for treatment of a disease associated with dysregulation of AMPK or dysregulation of an AMPK-associated pathway (*e.g.*, non-insulin dependent diabetes mellitus, dyslipidemia, etc.)

Therefore, contemplated pharmaceutical compositions will comprise at least one cytokinin or cytokinin analog in a formulation for treatment of non-insulin dependent diabetes mellitus and/or dyslipidemia, with the proviso that the cytokinin or cytokinin analog is a compound other than metformin, glyburide, AICAR, 1-morpholino-3-phenylurea, or rosiglitazone. As above, particularly contemplated pharmaceutical compositions will include one or more cytokinin or cytokinin analog as contemplated above.

In yet another aspect of the inventive subject matter, the inventors contemplate that identification of a compound as a cytokinin or cytokinin analog may be employed as one step in a method of producing a pharmaceutical composition for administration to a human. Suitable steps for the identification may include various test methods (*e.g.*, AMPK kinase assay, CRE1-binding assay, *in vivo* growth enhancement assay, or *in vitro* growth enhancement assay) as well as computational methods in which known cytokinin analogs are employed as a template for synthetic cytokinins and cytokinin analogs, or in which the binding structure of AMPK (or CRE1 or other cytokinin binding molecule) is used in a molecular docking algorithm. So identified compounds may then be included into the pharmaceutical composition.

Where contemplated compounds are employed as a dietary/nutritional supplement for human consumption, it should be particularly recognized that such nutritional supplements will comprise a cytokinin or cytokinin analog in an amount effective to reduce at least one of a blood glucose concentration, a triglyceride concentration, and a cholesterol concentration. In more preferred aspect of such supplements, the cytokinin or cytokinin analog is present in an amount that will decrease the blood concentration, triglyceride concentration, and/or cholesterol concentration at least 5%, and more typically at least 10% when the supplement is ingested over a period of 14 days, more typically 30 days, and most typically 60 days.

Further especially preferred supplements will include cytokinins and/or cytokinin analogs in which the cytokinin and/or cytokinin analog is a constituent of a plant extract (*e.g.*, prepared from barley or wheat as described in our copending patent application with the serial number 09/802,349, which was filed 03/08/01, and which is incorporated by reference herein).

Consequently, a method of marketing a product (*e.g.*, pharmaceutical composition, food item, or nutritional supplement) may include one step in which information is provided (*i.e.*, in which it is advertised) that a cytokinin or a cytokinin analog reduces blood glucose concentration, serum triglyceride concentration, and/or serum cholesterol concentration, and/or that the cytokinin or a cytokinin analog activates AMPK and/or an AMPK-associated pathway. The term "AMPK-associated pathway" as used herein refers to any signal pathway (and most typically a signal pathway that uses kinases) in which an element receives from, and/or provides to AMPK a signal.

In yet further contemplated aspects, a pharmaceutical composition may include an anticytokinin in a formulation for treatment of a disease associated with an overactivity of AMPK or an overactivity of an AMPK-associated pathway. Especially contemplated anticytokinins include 4-N-substituted-2-S-substituted pyrrolo[2,3-d]pyrimidines, but all other compounds that at least partially inhibit a cytokinin in a cytokinin assay as described above are also contemplated. Contemplated diseases include ventricular tachyarrhythmia, various neoplastic diseases, and especially those with solid tumors.

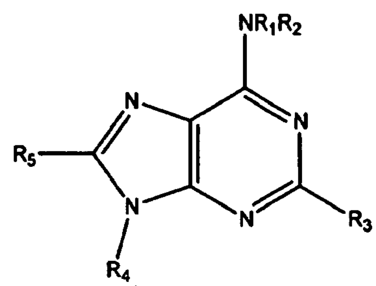
Thus, specific embodiments and applications of cytokinins and cytokinin analogs as therapeutic agents have been disclosed. It should be apparent, however, to those skilled in the art that many more modifications besides those already described are possible without departing from the inventive concepts herein. The inventive subject matter, therefore, is not to be restricted except in the spirit of the appended claims. Moreover, in interpreting both the specification and the claims, all terms should be interpreted in the broadest possible manner consistent with the context. In particular, the terms "comprises" and "comprising" should be interpreted as referring to elements, components, or steps in a non-exclusive manner, indicating that the referenced elements, components, or steps may be present, or utilized, or combined with other elements, components, or steps that are not expressly referenced.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising at least one cytokinin or cytokinin analog at a concentration effective to activate AMPK or to activate an AMPK-associated pathway in a mammal when the composition is administered to the mammal.

2. The pharmaceutical composition of claim 1 comprising a cytokinin having a structure according to Formula (I)



Formula (I)

wherein R_1 and R_2 are independently H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted alkaryl, optionally substituted heteroaryl, optionally substituted heterocycle, OH, NOH, CN, or NR_3R_4; and

wherein R_3, R_4, and R_5 are independently H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted alkaryl, optionally substituted heteroaryl, optionally substituted heterocycle, NH_2, OH, NOH, CN, CF_3, O-alkyl, S-alkyl, NH-alkyl, or a carbohydrate radical.

3. The pharmaceutical composition of claim 1 comprising a cytokinin analog.

4. The pharmaceutical composition of claim 3 wherein the cytokinin analog comprises a substituted thiourea, a N-monosubstituted urea, or N,N'-disubstituted urea.

5. The pharmaceutical composition of claim 4 wherein the cytokinin analog is a N-(4-pyridazinyl)urea, or a N-(2,6-disubstituted 4-pyridyl)-N'-phenylurea.

6. The pharmaceutical composition of claim 3 wherein the cytokinin analog comprises a substituted nitroguanidine.

7. The pharmaceutical composition of claim 6 wherein the cytokinin analog is substituted with an optionally substituted alkyl, alkenyl, or alkynyl radical.

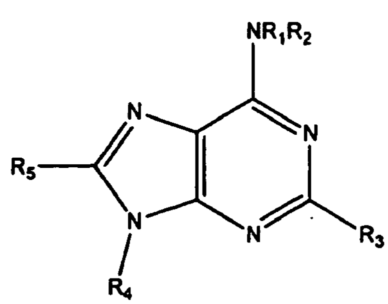
8. The pharmaceutical composition of claim 3 wherein the cytokinin analog comprises a substituted ethanolamine, a substituted oligo(imino-amine), or a substituted sulfonamide.

9. The pharmaceutical composition of claim 1 wherein administration is indicated for treatment of a disease associated with dysregulation of AMPK or dysregulation of an AMPK-associated pathway.

10. The pharmaceutical composition of claim 9 wherein the disease is non-insulin dependent diabetes mellitus, or a dyslipidemia.

11. A pharmaceutical composition comprising at least one cytokinin or cytokinin analog in a formulation for treatment of non-insulin dependent diabetes mellitus or a dyslipidemia, with the proviso that the cytokinin or cytokinin analog is a compound other than metformin, glyburide, AICAR, 1-morpholino-3-phenylurea, and rosiglitazone.

12. The pharmaceutical composition of claim 11 wherein the cytokinin or cytokinin analog has a structure according to Formula (I)



Formula (I)

wherein R_1 and R_2 are independently H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted alkaryl, optionally substituted heteroaryl, optionally substituted heterocycle, OH, NOH, CN, or NR_3R_4; and

wherein R_3, R_4, and R_5 are independently H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted alkaryl, optionally substituted heteroaryl, optionally substituted heterocycle, NH_2, OH, NOH, CN, CF_3, O-alkyl, S-alkyl, NH-alkyl, or a carbohydrate radical.

13. The pharmaceutical composition of claim 11 wherein the cytokinin analog comprises a substituted thiourea, a N-monosubstituted urea, or N,N'-disubstituted urea.

13. The pharmaceutical composition of claim 11 wherein the cytokinin analog comprises a substituted nitroguanidine.

14. The pharmaceutical composition of claim 11 wherein the cytokinin analog comprises a substituted ethanolamine, a substituted oligo(imino-amine), or a substituted sulfonamide.

15. The pharmaceutical composition of claim 11 wherein the formulation is for oral administration.

16. A method of producing a pharmaceutical composition for administration to a human, comprising:

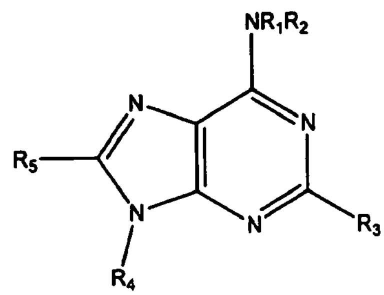
identifying a compound as having cytokinin activity; and

incorporating the compound into the pharmaceutical composition.

17. The method of claim 16 wherein the step of identifying includes at least one of an AMPK kinase assay, a CRE1-binding assay, an *in vivo* growth enhancement assay, an *in vitro* growth enhancement assay, and correlation of the compound with one or more known cytokinin or cytokinin analogs.

18. The method of claim 16 wherein the compound comprises a substituted thiourea, a N-monosubstituted urea, a N,N'-disubstituted urea, a substituted nitroguanidine, a substituted ethanolamine, a substituted oligo(imino-amine), or a substituted sulfonamide.

19. The method of claim 16 wherein the compound has a structure according to Formula (I)



Formula (I)

wherein R_1 and R_2 are independently H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted alkaryl, optionally substituted heteroaryl, optionally substituted heterocycle, OH, NOH, CN, or NR_3R_4; and

wherein R_3, R_4, and R_5 are independently H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted alkaryl, optionally substituted heteroaryl, optionally substituted heterocycle, NH_2, OH, NOH, CN, CF_3, O-alkyl, S-alkyl, NH-alkyl, or a carbohydrate radical.

20. The method of claim 16 further comprising a step of providing information that the compound activates AMPK or an AMPK-associated pathway.

21. A nutritional supplement comprising an at least partially isolated cytokinin or cytokinin analog in an amount effective to reduce at least one of a blood glucose concentration, a triglyceride concentration, and a cholesterol concentration.

22. The nutritional supplement of claim 21 wherein the at least partially isolated cytokinin or cytokinin analog is present as a constituent of a plant extract.

23. The nutritional supplement of claim 22 wherein the plant extract is prepared from barley.

24. A method of marketing a product comprising one step in which it is advertised that a cytokinin or a cytokinin analog reduces at least one of a blood glucose concentration, a triglyceride concentration, and a cholesterol concentration, or activates at least one of an AMPK or an AMPK-associated pathway.

25. The method of claim 24 wherein the cytokinin or cytokinin analog is present as a constituent of a plant extract.

26. The method of claim 24 wherein the cytokinin or cytokinin analog is prepared from a plant.

27. The method of claim 24 wherein the cytokinin or cytokinin analog is synthetic.

28. The method of claim 24 wherein the product is a pharmaceutical composition, a food item, or a nutritional supplement.

29. A pharmaceutical composition comprising an anticytokinin in a formulation for treatment of a disease associated with an overactivity of AMPK or an overactivity of an AMPK-associated pathway.

30. The pharmaceutical composition of claim 29 wherein the anticytokinin comprises a 4-N-substituted-2-S-substituted pyrrolo[2,3-d]pyrimidine.

31. The pharmaceutical composition of claim 29 wherein the disease is ventricular tachyarrhythmia.

ABSTRACT

Cytokinins and/or cytokinin analogs are employed as therapeutic agents for use in various mammals, and especially in human, wherein such compounds are employed to activate AMPK and/or to activate an AMPK associated pathway. Especially contemplated uses will therefore be directed to treatment of AMPK related conditions and diseases, including NIDDM, metabolic syndrome, and dyslipidemia.

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